MOLECULAR PHARMACOLOGY, 17, 295–300

Calcium Regulation by the Low-Affinity Taurine Binding Sites of Cardiac Sarcolemma

JAMES P. CHOVAN,* ELLIOTT C. KULAKOWSKI,* STEVEN SHEAKOWSKI* AND STEPHEN W. SCHAFFER*†

Center for Health Sciences, Division of Biochemistry and Biophysics, Lehigh University, Bethlehem, Pennsylvania 18015, and †Department of Physiology and Biophysics, Hahnemann Medical College, Philadelphia, Pennsylvania 19102

Received October 4, 1979; Accepted December 27, 1979

SUMMARY

CHOVAN, J. P., E. C. KULAKOWSKI, S. SHEAKOWSKI AND S. W. SCHAFFER. Calcium regulation by the low-affinity taurine binding sites of cardiac sarcolemma. *Mol. Pharmacol.* 17: 295–300 (1980).

Several lines of evidence are presented indicating that taurine binding to the low-affinity sites of cardiac sarcolemma is responsible for many previously observed, taurine-mediated pharmacological effects. First, verapamil inhibits taurine binding to the cell membrane in a dose-dependent manner. Second, verapamil reverses taurine enhancement of Ca^{2+} binding to the sarcolemma at concentrations at which verapamil itself has no significant effect on Ca^{2+} binding. Third, maximal reversal of the negative inotropic effect of both low Ca^{2+} and verapamil perfusion occurs at taurine concentrations above the apparent $K_{0.5}$ of the low-affinity sites. Fourth, hypotaurine is a potent inhibitor of taurine binding to the low-affinity sites and exerts taurine-like pharmacological effects. This is contrasted with two less potent inhibitors of taurine binding which possess no significant pharmacological activity. It is concluded that binding to low-affinity sarcolemmal sites is a fundamental step in the mechanism underlying the actions of taurine on the heart. The possibility that low-affinity taurine binding affects the Na^+/Ca^{2+} exchange system of the sarcolemma is discussed.

INTRODUCTION

Taurine (2-aminoethanesulfonic acid) is an ubiquitous amino acid making up approximately 50% of the free amino acid pool in mammalian heart (1). Interest in the study of taurine in the heart has been stimulated by two observations: (i) Taurine acts as an antiarrhythmic agent (2) and (ii) its tissue levels are altered during heart failure (3, 4).

We have previously reported that taurine has a slight, positive inotropic effect on perfused rat hearts (5), which we propose is related to its ability to enhance calcium binding to the sarcolemma (6). In the latter study, taurine was also seen to antagonize the inhibitory effects of verapamil on Ca^{2+} binding to the cell membrane. Since verapamil is highly lipophilic, its mechanism appears to involve a direct interaction with the sarcolemma (7).

Taurine has also been shown to interact directly with both high- and low-affinity binding sites on the sarcolemma (8). The high-affinity sites appear to be associated with the β-amino acid transport system since the $K_{0.5}$ for binding and the K_m of transport are approximately the same (8, 9). The role of the low-affinity sites, however, remains unclear. It is known that many of the reported actions of taurine on the heart (2, 5, 6, 10, 11) occur at concentrations at or above the apparent $K_{0.5}$ of these low-affinity sites (12).

Since taurine and verapamil both interact with the sarcolemma and apparently have antagonistic effects, we have utilized this interaction to define further the role of the low-affinity taurine binding sites. The data support our hypothesis that many of the reported actions of taurine on the heart are mediated by the interaction of this amino acid with its low-affinity binding sites.

MATERIALS AND METHODS

Hearts were removed from male Wistar rats (240–280 g) after decapitation and perfused within 45 s with Krebs–Henseleit buffer supplemented with 5 mM glucose and 2.5 U/liter insulin. The perfusate was gassed with a mixture of 95% O_2/5% CO_2 to maintain a pH of 7.4.

The standard working heart apparatus employed was a modified version of that described by Neely *et al.* (13) and used in previous studies (5, 14, 15). Cardiac output was determined by a flowmeter placed above the aortic cannula, and left ventricular pressure was measured with a Statham P23Gb pressure transducer by inserting a 22-gauge needle through the ventricle wall. Pressure work was calculated according to Neely *et al.* (13).

Preliminary studies indicated that 0.4 mM Ca^{2+} was

This work was supported in part by U. S. Public Health Service Grant HL-18554.

0026-895X/80/030295-06$02.00/0

the maximum perfusate calcium concentration which produced a nonworking heart. All Ca^{2+} titration studies were, therefore, begun at this concentration. Calcium levels were incrementally raised by the addition of $CaCl_2$ to the perfusate in the presence and absence of 10 mM taurine, 3×10^{-8} M verapamil, or both. The hearts were allowed to stabilize to new steady-state levels of left ventricular pressure and output before further additions. This experiment was also performed in the presence of several taurine analogs.

In the preparation of cardiac sarcolemma, rat hearts were perfused with normal buffer for 5 min to remove blood from the coronary arteries and ventricles. Ventricular sarcolemma was isolated according to Sulakhe *et al.* (16). Taurine (8) and calcium (5) binding assays were performed using the Millipore filtration system described previously.

Both ^{14}C-taurine, shown to be radiochromatographically pure by thin-layer chromatography, and $^{45}CaCl_2$ were obtained from New England Nuclear. Unlabeled taurine was obtained from Aldrich Chemical Company and recrystallized twice from water prior to use. Verapamil was a gift of Knoll Pharmaceutical Company, Whippany, New Jersey. Ultrapure Tris was a gift of Henry Ziegler, Chemzymes, Inc., East Stroudsburg, Pennsylvania. Wistar rats were purchased from Ace Animals, Inc. All other reagents were purchased from Fisher Scientific Company.

RESULTS

Taurine and verapamil have previously been shown to exhibit antagonistic effects on Ca^{2+} binding to cardiac sarcolemma (6). To examine if this antagonism is caused by direct inhibition of taurine binding to the low-affinity sites by verapamil, sarcolemma were incubated with 3.5 mM taurine and varying concentrations of verapamil. As seen in Fig. 1, verapamil concentrations over the range of 10^{-10} to 10^{-6} M cause a progressive decline in taurine binding to 20% of control. Since taurine binding, but not

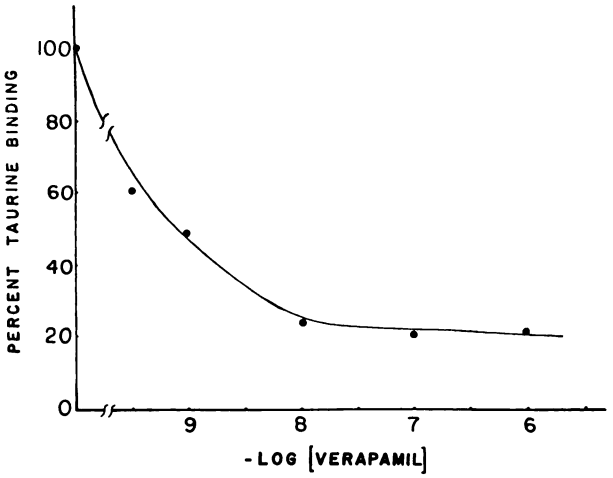

FIG. 1. *Verapamil inhibition of taurine binding to isolated cardiac sarcolemma*

Ventricular sarcolemma were isolated and assayed for taurine binding as described in Materials and Methods. Sarcolemma were incubated with 3.5 mM ^{14}C-taurine and varying concentrations of verapamil from 10^{-10} to 10^{-6} M. Values shown are the means of three assays.

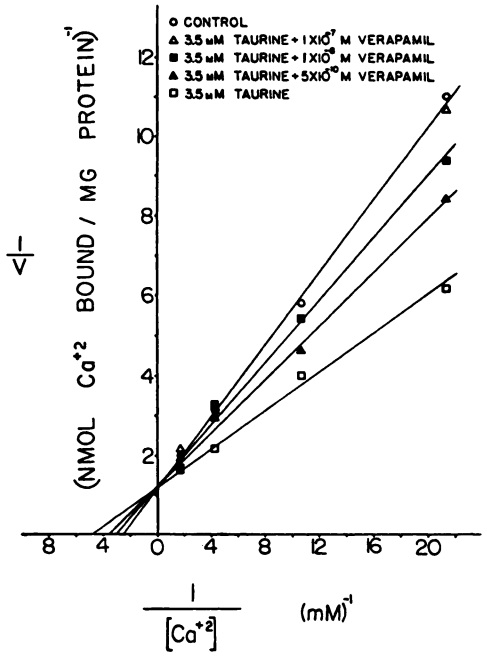

FIG. 2. *Double-reciprocal Scatchard plot of calcium binding to isolated cardiac sarcolemma in the presence of taurine and varying concentrations of verapamil*

Ventricular sarcolemma were isolated and assayed for Ca^{2+} binding as described in Materials and Methods. Sarcolemma were assayed in the presence and absence of 3.5 mM taurine or with medium containing 3.5 mM taurine and verapamil concentrations varying from 5×10^{-10} to 10^{-7} M. Under all conditions, Ca^{2+} concentrations were varied from 0.04 to 0.8 mM. Values shown are the means of four assays.

Ca^{2+} binding,[1] is inhibited by concentrations of verapamil below 10^{-8} M, verapamil is a useful tool in examining the role of the taurine binding sites. The effect of taurine on calcium binding in the presence and absence of varying concentrations of verapamil is shown in Fig. 2. The data were analyzed using a double-reciprocal Scatchard plot in which a change in the X axis indicates alterations in the K_a of Ca^{2+} for its membrane receptors, while a change in the Y axis reveals a modification in the number of binding sites (Fig. 2). Consistent with previous data (6), 3.5 mM taurine was found to increase the affinity of some sites for Ca^{2+} without altering the overall number of binding sites on the membrane. As the concentration of verapamil was increased from 5×10^{-10} to 10^{-7} M, there was an apparent competitive, dose-dependent inhibition of taurine-enhanced Ca^{2+} binding. Thus, it appears as if verapamil-mediated reversal of the taurine effect is due at least in part to its inhibition of taurine binding to the low-affinity sites on the sarcolemma.

Competition between taurine and verapamil is also observed in the isolated, perfused rat heart exposed to varying concentrations of extracellular calcium. As seen in Fig. 3, a sigmoidal calcium titration curve was obtained; the addition of 10 mM taurine displaced this work curve to lower values of calcium, indicating that taurine increases the sensitivity of the heart to extracellular calcium. In contrast, the calcium concentration required for half-maximal work output increased from 0.8 to 2.0

[1] Unpublished observations.

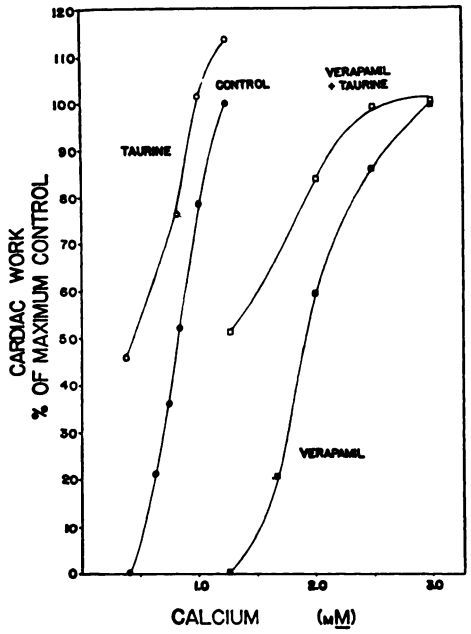

FIG. 3. *Calcium titration studies on perfused heart in the presence of taurine and verapamil*

Perfusion was begun at 0.4 mM Ca^{2+}, and hearts were allowed to stabilize before further addition of $CaCl_2$ (see Materials and Methods). Perfusate Ca^{2+} concentration was progressively increased and the work output monitored in control hearts or hearts perfused with 10 mM taurine, 3×10^{-8} M verapamil, or both 10 mM taurine and 3×10^{-8} M verapamil. Values shown are the means of three to seven hearts.

mM in the presence of 3×10^{-8} M verapamil. The inhibitory effect of verapamil was partially overcome by taurine as noted by the displacement of the half-maximal work output concentration from 2.0 to 1.25 mM Ca^{2+}. These data are consistent with the studies of Guidotti and Giotti (17) and reveal that the effects of taurine are greatest in hearts made hypodynamic by perfusion with buffer containing either a low calcium concentration or a cardiodepressant. They are also consistent with the view that a competition exists between taurine and verapamil at the low-affinity taurine sites.

Another approach employed to examine the role of the low-affinity sites was the use of taurine analogs. If the actions of taurine are mediated by these sites, then analogs of taurine with the same electronic and stereochemical configuration would be expected to inhibit taurine binding to the low-affinity protein and also alter sarcolemmal calcium binding and myocardial contractility. Conversely, analogs which fail to interact with the low-affinity sites should not mimic the pharmacological effects of taurine. Figure 4 shows the results of two taurine analogs; β-alanine, the carboxylic acid analog of taurine, has no effect on the low-affinity taurine binding protein even at 10-fold higher concentrations. This indicates that the nature of the negative group is an important factor in determining the strength of the interaction with the low-affinity binding sites. On the other hand, hypotaurine, the sulfinic acid analog of taurine, is a potent inhibitor of taurine binding even at lower concentrations (Fig. 4 and Ref. 8).

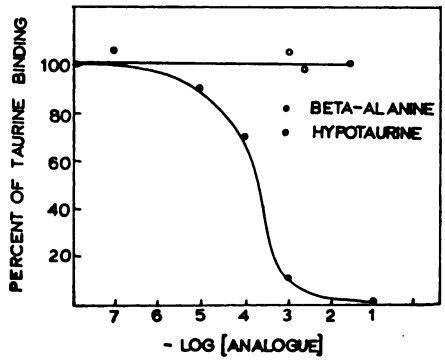

FIG. 4. *Analog inhibition of taurine binding to isolated cardiac sarcolemma*

Ventricular sarcolemma were isolated and assayed for taurine binding as described in Materials and Methods. Sarcolemma were incubated with 1.4 mM ^{14}C-taurine and varying concentrations of either hypotaurine or β-alanine from 10^{-7} to 10^{-1} M. Values shown are the means of three assays.

TABLE 1

Specificity of low-affinity taurine binding sites

Cardiac sarcolemma were isolated and taurine binding was performed as described in Materials and Methods. ^{14}C-Taurine (3.5 mM) was incubated in the presence and absence of equimolar concentrations of the listed taurine analogs. Each value presented is the average of three assays and is expressed as the percentage of control. An asterisk indicates a significant difference from the control ($P < 0.01$, using the paired Student's *t* test).

Taurine analog	% Taurine binding
Control	100
L-Methionine	110
L-Valine	57*
L-Aspartate	107
L-Asparagine	117
L-Glutamine	122
L-Leucine	62*
L-Cysteine	135
β-Alanine	124
Isethionic acid	57*
Hypotaurine	0*
Cysteine sulfinic acid	74

Table 1 further shows the specificity of these binding sites. At equimolar concentrations (3.5 mM), only L-valine, L-leucine, and isethionic acid significantly affect taurine binding. Consistent with Fig. 4, β-alanine is seen to have essentially no interaction with the taurine sites, while equimolar hypotaurine completely inhibited taurine binding.

If taurine mediates its action through the low-affinity sites, based on its inhibition data one would expect that hypotaurine would mimic the actions of taurine, while β-alanine and isethionic acid should exhibit little or no taurine-like activity. This was confirmed, as shown in Figs. 5 and 6. Figure 5 depicts the results of Ca^{2+} titration studies in the presence and absence of 10 mM taurine or an equal concentration of one of its analogs. β-Alanine is seen to have a slight, but insignificant effect on cardiac work over the Ca^{2+} concentration range studied, while isethionic acid appears to have no observable activity. In accord with its effect on low-affinity taurine binding, hypotaurine stimulates cardiac work to the same degree as taurine over the Ca^{2+} concentration range studied.

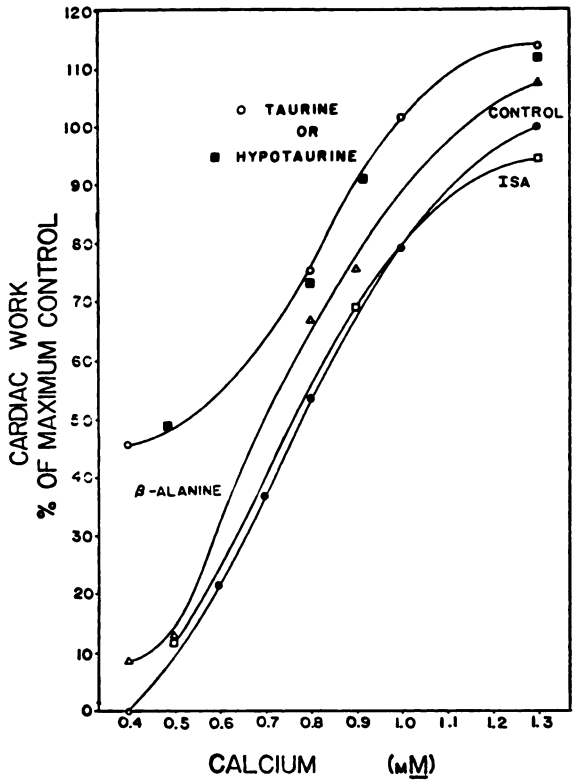

FIG. 5. *Calcium titration studies on perfused heart in the presence of taurine analogs*

Perfusion was carried out as described for Fig. 3. The concentration of taurine or its analogs used was 10 mM. Values shown are the means of three to seven hearts.

The hypothesis is also supported by sarcolemmal calcium binding studies. Figure 6A shows the binding profiles for Ca^{2+} to sarcolemma which was incubated in a medium containing either taurine or one of its analogs. In the presence of 0.5 mM hypotaurine, Ca^{2+} binding is significantly enhanced (Fig. 6A), while neither 10 mM β-alanine nor 10 mM isethionic acid had any effect. Consistent with its higher affinity for the low-affinity sites (Fig. 4), hypotaurine potentiation of Ca^{2+} binding occurs at a lower concentration than the level of taurine required to mediate similar effects; 1.0 mM taurine has a minimal effect on both Ca^{2+} binding and myocardial contraction.[1] Moreover, the effects of 0.5 mM hypotaurine and 3.5 mM taurine, the apparent K_i and $K_{0.5}$ for taurine binding, respectively, are comparable. Likewise, complete saturation of these sites, which occurs at a concentration of 10 mM, yields a maximal response regardless of which analog is employed.

Scatchard analysis of the Ca^{2+} binding isotherms has been interpreted as showing the existence of at least two classes of Ca^{2+} binding sites (6). Hypotaurine (0.5 mM) was found to affect primarily the low-affinity sites, whereas 10 mM taurine or 10 mM hypotaurine potentiated Ca^{2+} binding to both classes of sites (Fig. 6B). Thus, the effects of taurine and hypotaurine on Ca^{2+} binding are specific, saturable, and dose dependent, as would be expected if their actions were mediated through a binding protein.

DISCUSSION

It is becoming increasingly apparent that taurine regulates Ca^{2+} homeostasis in the heart. Evidence presented here supports this view. Taurine was found to exhibit a positive inotropic effect on perfused rat hearts made hypodynamic by either a reduction in extracellular calcium or treatment with the cardiodepressant verapamil. Our data are consistent with similar studies performed with guinea pig heart (11), but are at variance with the rat heart studies of Dietrich and Diacono (10), in which taurine potentiation of the negative inotropic effect of reduced extracellular calcium was reported. However, further agreement with our results comes from the observation that taurine delays the loss of myocardial Ca^{2+} and contractile force during low-Ca^{2+} perfusion in both the rat and guinea pig (17, 18).

Recently, taurine administration has also been linked to reductions in tissue Ca^{2+} levels. McBroom and Welty (19) found that taurine prevents the 12-fold increase in

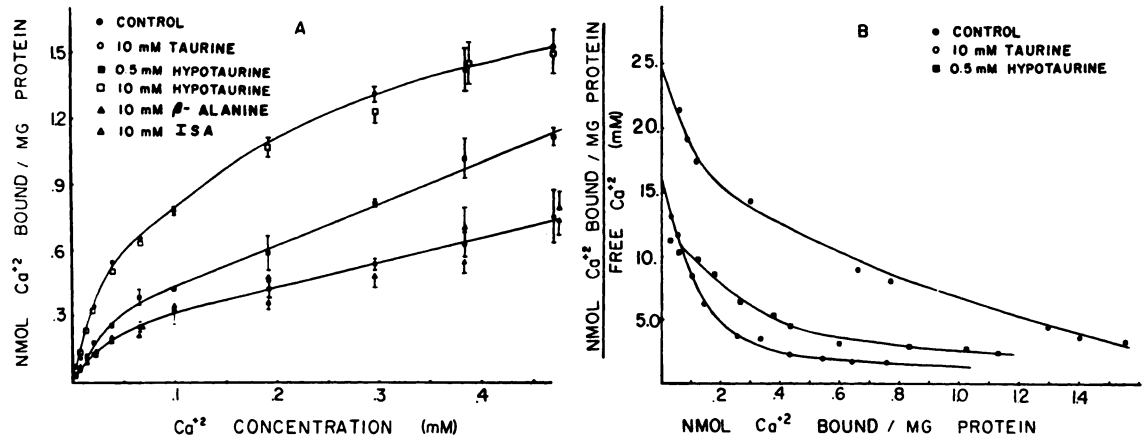

FIG. 6. *Calcium binding to isolated cardiac sarcolemma in the presence of taurine analogs*

Ventricular sarcolemma were isolated and assayed for Ca^{2+} binding as described in Materials and Methods. The membrane was incubated in a medium containing 100 mM KCl, 5 mM NaCl, 10 mM $MgCl_2$, 20 mM Tris-maleate, and varying $^{45}CaCl_2$ concentrations from 3.0 to 500 μM. When present, taurine or one of its analogs was at the concentration stated in the figure. ISA is isethionic acid. (A) Binding profile. (B) Scatchard analysis of the data in A. Isethionic acid, β-alanine, and 10 mM hypotaurine are omitted for clarity. Values shown are the means ± SEM of four assays.

intracellular calcium content which occurs in congenital cardiomyopathic hamsters, while we reported that taurine protects the heart against Ca^{2+} overload which occurs after a specific period of calcium-free perfusion (20).

Correlated with the positive inotropic effect of taurine is the observation that taurine stimulates calcium binding to isolated heart sarcolemma (6). Moreover, a relationship exists between the prevention of calcium overload and a reduction in excessive calcium binding to the sarcolemma (20). These data suggest that the effects of taurine result in part from its actions on the cell membrane.

Sarcolemma isolated from rat and swine heart have been shown to contain two taurine binding proteins (8, 12). Both of the proteins from pig heart are characterized by sigmoidal binding isotherms, while only the low-affinity protein has this pattern in the rat. Each binding component also exhibits an affinity for concanavalin A, suggesting that they are glycoproteins. This indicates that both factors are located on the extracellular surface of the sarcolemma, although they may also be transmembrane proteins. In fact, the high-affinity protein most likely spans the membrane, since it appears to be associated with the β-amino acid uptake system (8, 9).

In this study several lines of evidence have been provided implicating the low-affinity taurine binding sites of cardiac sarcolemma in the actions of taurine. First, the taurine-mediated pharmacological effects are observed at or above the apparent $K_{0.5}$ of taurine binding to these sites. Second, there is a correlation between the concentration of taurine required to saturate the binding sites and the concentration necessary to maximize its dose-dependent effects. Third, the taurine analog hypotaurine interacts with the low-affinity sites and mimics the pharmacological effects of taurine on sarcolemmal calcium binding and contractility. Conversely, β-alanine and isethionate have a greatly reduced affinity for the low-affinity sites and fail to mediate these taurine-like pharmacological effects. Finally, reversal by verapamil of the taurine-mediated changes in both contraction and Ca^{2+} binding seems to result in part from inhibition of taurine binding to the low-affinity sites. Since there are about four times as many low-affinity taurine binding sites as high-affinity sites on rat heart sarcolemma (8), the failure of verapamil to decrease taurine binding more than 80% (Fig. 1) suggests that verapamil preferentially inhibits low-affinity taurine binding.

The mechanism by which taurine interaction with the low-affinity sites causes changes in calcium binding to the sarcolemma remains unclear. Previous attempts to demonstrate taurine-induced conformational changes in the membrane failed (6). However, the use of verapamil afforded us a new means to probe the actions of taurine. Verapamil was found by ESR to have no effect on the rotational correlation coefficient of the spin label 2N14, but did appear to increase spin–spin interactions. One interpretation of these results is that verapamil alters the aggregation state of the label, indicating that it changes membrane fluidity. Since this effect was absent in the presence of taurine, the amino acid appears to reverse the verapamil-induced membrane changes.

Another interpretation of the data is the existence of a competitive interaction between taurine and verapamil for a particular component of the sarcolemma. This is strongly suggested by the observed effects of low verapamil concentrations on taurine-enhanced Ca^{2+} binding (Fig. 2). In both retinal synaptosomes and rod outer segments, taurine appears to inhibit Ca^{2+} accumulation by interacting with a Na^+/Ca^{2+} exchange system (21). Recently, Langer *et al.* (22) have proposed that verapamil interacts with a similar system on the cardiac sarcolemma. They have further suggested that this system is responsible for providing the necessary Ca^{2+} required for maximal contraction. If taurine likewise affects this exchange in the heart, this would account for all of the observed pharmacological effects of taurine, as well as the apparent competition between taurine and verapamil. Studies are presently underway to isolate and fully characterize both taurine binding components and to determine their relationship to the Na^+/Ca^{2+} exchange system.

ACKNOWLEDGMENTS

The authors wish to thank Jay Kramer for his technical assistance and Mary Ann Elgin for the typing of the manuscript.

REFERENCES

1. Jacobsen, J. G. and L. H. Smith, Jr. Biochemistry and physiology of taurine and taurine derivatives. *Physiol. Rev.* **48:** 424–511 (1968).
2. Read, W. O. and J. D. Welty. Effect of taurine on epinephrine and digoxin induced irregularities of the dog heart. *J. Pharmacol. Exp. Ther.* **139:** 283–289 (1963).
3. Peterson, M. B., R. J. Mead and J. D. Welty. Free amino acids in congestive heart failure. *J. Mol. Cell. Cardiol.* **5:** 139–147 (1973).
4. Newman, W. H., C. J. Frangakis, D. S. Grosso and R. Bressler. A relationship between myocardial taurine content and pulmonary wedge pressure in dogs with heart failure. *Physiol. Chem. Phys.* **9:** 259–263 (1977).
5. Schaffer, S. W., J. P. Chovan and R. F. Werkman. Dissociation of cAMP changes and myocardial contractility in taurine perfused rat heart. *Biochem. Biophys. Res. Commun.* **81:** 248–253 (1978).
6. Chovan, J. P., E. C. Kulakowski, B. W. Benson and S. W. Schaffer. Taurine enhancement of calcium binding to rat heart sarcolemma. *Biochim. Biophys. Acta* **551:** 129–136 (1979).
7. Bondi, A. Y. Effects of verapamil on excitation-contraction coupling in frog sartorius muscle. *J. Pharmacol. Exp. Ther.* **205:** 49–57 (1978).
8. Kulakowski, E. C., J. Maturo and S. W. Schaffer. The identification of taurine receptors from rat heart sarcolemma. *Biochem. Biophys. Res. Commun.* **80:** 936–941 (1978).
9. Huxtable, R. and J. Chubb. Adrenergic stimulation of taurine transport by the heart. *Science* **198:** 409–411 (1977).
10. Dietrich, J. and J. Diacono. Comparison between ouabain and taurine effects on isolated rat and guinea-pig hearts in low calcium medium. *Life Sci.* **10:** 499–507 (1971).
11. Dolara, P., A. Agresti, A. Giotti and G. Pasquini. Effect of taurine on calcium kinetics of guinea-pig heart. *Eur. J. Pharmacol.* **24:** 352–358 (1973).
12. Kulakowski, E. C., J. Maturo and S. W. Schaffer. Taurine binding to solubilized ventricular sarcolemma. *Fed. Proc.* **38:** 744 (1979).
13. Neely, J. R., H. Liebermeister, E. J. Battersby and H. E. Morgan. Effects of pressure development on oxygen consumption by isolated rat heart. *Am. J. Physiol.* **212:** 804–814 (1967).
14. Schaffer, S., B. Safer and J. R. Williamson. Investigation of the role of mitochondria in the cardiac contraction-relaxation cycle. *FEBS Lett.* **23:** 125–130 (1972).
15. Schaffer, S. W., B. Safer, A. Scarpa and J. R. Williamson. Mode of action of the calcium ionophores X-537A and A23187 on cardiac contractility. *Biochem. Pharmacol.* **23:** 1609–1617 (1974).
16. Sulakhe, P. V., G. I. Drummond and D. C. Ng. Calcium binding by skeletal muscle sarcolemma. *J. Biol. Chem.* **248:** 4150–4157 (1973).
17. Guidotti, A. and A. Giotti. Taurine in the cardiovascular system. *Rec. Prog. Med. (Roma)* **49:** 61–71 (1970).
18. Chubb, J. and R. J. Huxtable. Transport and biosynthesis of taurine in the stressed heart, in *Taurine and Neurological Disorders.* (A. Barbeau and R. J. Huxtable, eds.). Raven Press, New York, 161–178 (1978).
19. McBroom, M. J. and J. D. Welty. Effects of taurine on heart calcium in the cardiomyopathic hamster. *J. Mol. Cell. Cardiol.* **9:** 853–858 (1977).

20. Chovan, J., J. Kramer and S. Schaffer. Protection by taurine of calcium paradox damage. *J. Mol. Cell. Cardiol.* (*Suppl. 1*) 11: 15 (1979).
21. Pasantes-Morales, H., R. M. Ademe and A. M. López-Colomé. The effect of taurine on ^{45}calcium transport by retinal sub-cellular fractions, in *Actions of Taurine on Excitable Tissues* (S. W. Schaffer, J. J. Kocsis and S. I. Baskin, eds.). Raven Press, New York, in press (1980).
22. Langer, G. A., J. S. Frank and A. J. Brady. The Myocardium, in *International Review of Physiology, Vol. 9, Cardiovascular Physiology II* (A. C. Guyton and A. W. Cowley, eds.). University Park Press, Baltimore, 191–238 (1976).

Send reprint requests to: Stephen W. Schaffer, Center for Health Sciences, Seeley G. Mudd Building 6, Lehigh University, Bethlehem, Pennsylvania 18015.